Section 1

Limit Tests

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LIMIT TESTS

The medicines for human use should be pure, safe and efficacious. The impure drugs may compromise the safety and efficacy. Indian pharmacopoeia has fixed permissive limits of tolerance for impurities in different drugs with prime consideration that the product should be satisfactory clinically. The limits set in pharmacopoeia depend upon

1. Intended use of the official compound.
2. Safety considerations.
3. Cost of production.

Adherence of the drug to official standards ensures consistent therapeutic response, acceptable level of potency and freedom from toxicity.

Different sources of impurities in pharmaceutical substance are:

1. Raw materials.
2. The manufacturing process: During the manufacturing process impurities can enter the final product through
   - The starting material and its impurities.
   - Intermediates.
   - Reagents, solvents and catalysts used in the process.
   - Metal of the reaction vessel.
3. Chemical decomposition in presence of air, light and moisture leading to hydrolysis, oxidation, etc. of the drug.
4. Improper storage and deliberate adulteration.

In this part we are going to perform the limit tests for chlorides, sulphates, arsenic, iron and heavy metals. Limit tests are quantitative or semiquantitative tests designed to identify and control small quantity of impurities which are likely to be present in the substance. Arsenic and heavy metals have cumulative undesirable effect in the body and thus rigid limits are set for them, in substances intended for internal use.

In limit test for chlorides, sulphates, iron and heavy metals a pair of Nessler cylinders in used. Nessler cylinders are matched tubes of clear, colourless, lead-free glass with uniform internal diameter and a flat, transparent base. They are of 50 ml capacity with 150 mm overall height. The external height to 50 ml mark is around 110 to 124 mm. The thickness of wall is about 1.0 to 1.5 mm. The thickness of the base is around 1.5 to 3.0 mm. The height to 50 ml mark in two cylinders should not vary by >1 mm.

THEORY AND CHEMISTRY INVOLVED

When silver nirate (in presence of nitric acid) is added to the sample or test solution, it reacts with chlorides precipitating silver chloride.

$$\underset{\text{Silver nitrate}}{AgNO_3} + \underset{\text{Chloride ion}}{Cl^-} \xrightarrow{HNO_3} \underset{\text{Silver chloride (white ppt)}}{AgCl\downarrow} + \underset{\text{Nitrate ion}}{NO_3^-}$$

The opalescence obtained with sample is compared with standard opalescence produced from a definite quantity of chloride ions in the form of sodium chloride. Both the cylinders are viewed transversely against a dark background.

Calculations

If the degree of opalescence is same in the test and standard solution then

1.25 g of sample ≅ 10 ml of 25 ppm chloride

1.25 g of sample ≅ 0.00025 g of chloride

1 g of sample $\cong \dfrac{0.00025 \text{ g of chloride}}{1.25}$

≅ 0.0002 g of chloride

1000000 g of sample ≅ 200 g of chloride

1 g of sample ≅ 200 ppm of chloride

Experiment 1: Limit Test for Chlorides

AIM

To perform the limit test for chlorides in the given sample of sodium bicarbonate.

CHEMICALS REQUIRED

Sodium bicarbonate, nitric acid, 0.1 M silver nitrate solution.

APPARATUS REQUIRED

Nesseler cylinders, measuring cylinder, beaker, glass rod.

PROCEDURE

Preparation of Test Opalescence (200 ppm)

Dissolve 1.25 g of sample in 15 ml of water and 2 ml of nitric acid. Transfer to the Nesseler cylinder. Dilute to 50 ml with water and add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes protected from light.

Preparation of Standard Opalescence

Take 10 ml of chloride standard solution (25 ppm Cl) in a Nessler cylinder. Mix it with 5 ml of water. Add 10 ml of dilute nitric acid. Dilute to 50 ml with water and add 1 ml of 0.1 M silver nitrate. Stir immediately with a glass rod and allow to stand for 5 minutes protected from light.

Observe transversely, the opalescence in two cylinders, against a black background.

OBSERVATION

Sample turbidity is _____ than the standard turbidity.

RESULT

Sample of sodium bicarbonate _____ the limit test for chloride.

THEORY AND CHEMISTRY INVOLVED

This test is designed to control and detect the sulphate impurity, if present in inorganic substances. The reaction involved is

$$\underset{\text{Potassium sulphate}}{K_2SO_4} + \underset{\text{Barium chloride}}{BaCl_2} \longrightarrow \underset{\text{Barium sulphate (white ppt)}}{BaSO_4\downarrow} + \underset{\text{Potassium chloride}}{2KCl}$$

The barium sulphate, prepared *in situ*, assists rapid and complete precipitation by seeding. Test solution contains a small amount of potassium sulphate to increase the sensitivity of the test. Sulphate ion impurity in the sample will produce barium sulphate in excess of already dissolved amount and produce turbidity. Small amount of ethanol is added to prevent supersaturation of barium sulphate. Acetic acid is used as acidifying agent.

Calculations

If the degree of test opalescence is equal to standard opalescence then

1 g of sample ≅ 15 ml of 10 ppm sulphate
1 g of sample ≅ 0.00015 g of SO_4
1000000 g of sample ≅ 150 g of SO_4
1 g of sample ≅ 150 ppm of SO_4

Experiment 2: Limit Test for Sulphates

Date _____

AIM

To perform the limit test for sulphates in the given sample of sodium bicarbonate.

CHEMICALS REQUIRED

Sodium bicarbonate, barium chloride, ethanol, potassium sulphate, 5 M acetic acid, hydrochloric acid.

APPARATUS REQUIRED

Nessler cylinders, measuring cylinder, beaker, glass rod.

PROCEDURE

Preparation of Test Opalescence (150 ppm)

Take 1.0 ml of a 25% w/v solution of barium chloride in a Nessler cylinder. Add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4), mix and allow to stand for 1 minute. In another beaker, suspend 1 g in 10 ml of distilled water, neutralise with hydrochloric acid and dilute to 15 ml with distilled water. Transfer the solutions to Nessler cylinder. Add sufficient water to produce 50 ml. Stir immediately with a glass rod and allow to stand for 5 minutes.

Preparation of Standard Opalescence

Take 1 ml of a 25% w/v solution of barium chloride in Nessler cylinder. Add 1.5 ml of ethanolic sulphate standard solution (10 ppm SO_4), mix and allow to stand for 1 minute. Add 15 ml of sulphate standard solution (10 ppm SO_4) and 0.15 ml of 5 M acetic acid. Add sufficient water to produce 50 ml, stir immediately with a glass rod and allow to stand for 5 minutes.

Observe both cylinders transversely against the black background.

Preparation of Ethanolic Sulphate Standard Solution (10 ppm SO_4)

Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in ethanol (30%) to 100 volumes with 30% ethanol.

Preparation of Sulphate Standard Solution (10 ppm SO_4)

Dilute 1 volume of a 0.181% w/v solution of potassium sulphate in distilled water to 100 volumes with the same solvent. It contains 0.00001 g SO_4/ml.

OBSERVATION

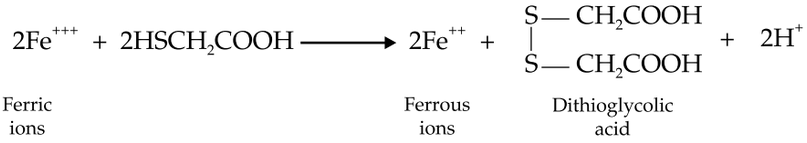
Sample turbidity is _____ than the standard turbidity.

RESULT

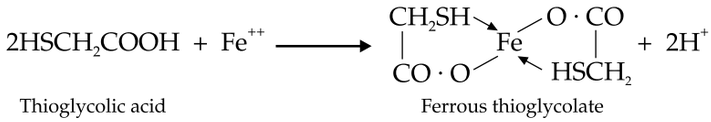
Sample of sodium bicarbonate _____ the limit test for sulphates.

THEORY AND CHEMISTRY INVOLVED

In this test purple colour is produced by reaction between thioglycolic acid (mercaptoacetic acid) and iron, in a solution made alkaline with ammonia and containing citric acid. Thioglycolic acid reduces fenic ions to ferrous ions.



Thioglycolic acid reacts with ferrous ions to form coordination compound ferrous thioglycolate which is purple in colour.



This ferrous thioglycolate formed is colourless in acidic medium but in alkaline medium it gives purple colour. Citric acid prevents precipitation of iron with ammonia and keeps iron in solution by forming a soluble complex with iron.

Possible Observations and Inferences

S. no.	*Observation*	*Inference*
1.	Sample colouration > standard colouration	Sample fails the limit test for iron
2.	Sample colouration < standard colouration	Sample passes the limit test for iron
3.	Sample colouration = standard colouration	Sample passes the limit test for iron

Calculations

If the intensity of sample colouration is same as that of standard colouration then

0.2 g of sample $\cong$ 2 ml of 20 ppm Fe^{+++}

0.2 g of sample $\cong$ 0.00004 g of Fe^{+++}

10,00,000 g of sample $\cong \dfrac{0.00004 \times 10,00,000}{0.2}$

1 g of sample $\cong$ 200 ppm of Fe^{+++}

Experiment 3: Limit Test for Iron

Date _____

AIM

To perform the limit test for iron in the given sample of calcium carbonate.

CHEMICALS REQUIRED

Calcium carbonate, citric acid, thioglycolic acid, ammonia solution, ferric ammonium sulphate, sulphuric acid, hydrochloric acid. All the reagents should be free from iron (except ferric ammonium sulphate). The reagents used in this test are designated as FeT.

APPARATUS REQUIRED

Nessler cylinders, measuring cylinder, beaker, glass rod.

PROCEDURE

Preparation of Test Solution (200 ppm)

Dissolve 0.2 g in 5 ml water and 0.5 ml of iron free hydrochloric acid, boil and dilute to 40 ml with water. Transfer to a Nessler cylinder. Add 2 ml of a 20% thioglycolic acid, mix, make alkaline with iron free ammonia solution. Dilute to 50 ml with water and allow to stand for 5 minutes.

Preparation of Standard Solution

Take 2.0 ml of iron standard solution (20 ppm Fe) in a Nessler cylinder. Add 40 ml of distilled water. Add 2 ml of a 20% solution of iron free citric acid and 0.1 ml of thioglycolic acid, mix, make alkaline with iron free ammonia solution. Dilute to 50 ml with water and allow to stand for 5 minutes.
Observe the pink-violet colouration produced in both Nessler cylinders.

Preparation of Iron Standard Solution (20 ppm Fe)

Dilute l volume of 0.1726% w/v solution of ferric ammonium sulphate in 0 .05 M sulphuric acid to 10 volumes with water.

OBSERVATION

Sample colouration is _____ than standard colouration.

RESULT

Sample of sodium bicarbonate _____ the limit test for iron.

THEORY AND CHEMISTRY INVOLVED

The limit for heavy metals is indicated in terms of the parts of lead (Pb), per million parts (by weight) of the substance being examined. This test is performed to determine the content of the metallic impurities in the sample. When metallic impurities react with hydrogen sulphide, coloured metal sulphides are precipitated.

The test is based on the formation of brown colour due to precipitation of metal sulphides at or about pH 3.5, in colloidal form.

$$\text{Metal} + H_2S \longrightarrow \underset{\text{(brown)}}{\text{Metal sulphide}} + H_2\uparrow$$

Intensity of the colour produced changes with the concentration of the metallic impurity. Concentrated solutions of lead salts produce black precipitate while dilute solutions produce brown precipitate. The test is performed by comparing the colour produced by standard lead solution and test solution.

Calculations

Sodium chloride is required to contain not > 5 ppm of heavy metals calculated as Pb. If the intensity of colour of test solution is equal to standard solution then

4 g of sodium chloride ≅ 1 ml of 20 ppm Pb
4 g of sodium chloride ≅ 0.00002 g of Pb
10,00,000 g of sodium chloride $\cong \frac{0.00002 \times 10,00,000}{4}$
1 g of sodium chloride ≅ 5 ppm of Pb

Experiment 4: Limit Test for Heavy Metals

Date _____

AIM

To perform the limit test for heavy metals in the given sample of sodium chloride.

CHEMICALS REQUIRED

Sodium chloride, dilute acetic acid, freshly prepared hydrogen sulphide solution, lead nitrate.

The reagents used should be free from heavy metals. They are designated as Sp.

APPARATUS REQUIRED

Nessler cylinders, beaker, measuring cylinder, glass rod.

PROCEDURE

Preparation of Test Solution (5 ppm)

Dissolve 4 g of sodium chloride in 2 ml of dilute acetic acid and add sufficient water to produce 25 ml. Transfer to a Nessler cylinder. Adjust the pH in between 3.0 and 4.0 with dilute acetic acid or dilute ammonia solution. Dilute with water to about 35 ml and mix. Add l0 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water. Allow to stand for five minutes.

Preparation of Standard Solution

Into a 50 ml Nessler cylinder, pipette 1.0 ml of lead standard solution (20 ppm Pb) and dilute with water to 25 ml. Adjust with dilute acetic acid or dilute ammonia solution to a pH between 3 .0 and 4.0, dilute with water to about 35 ml and mix. Add 10 ml of freshly prepared hydrogen sulphide solution, mix, dilute to 50 ml with water. Allow to stand for 5 minutes.

View both cylinders downwards over a white surface. Compare the colour produced with the test solution and standard solution.

Preparation of Lead Standard Solution (20 ppm)

Dissolve 0.400 g of lead nitrate in water containing 2 ml of nitric acid and add sufficient water to produce 250 ml (lead standard solution 0.1 % Pb). Take 1.0 ml of this solution and dilute to 10 ml (lead standard solution 100 ppm Pb). Take 1 ml of this solution and dilute to 5 ml with water to get lead standard solution 20 ppm Pb.

Hydrogen Sulphide Solution

A recently prepared saturated solution of hydrogen sulphide in water.

OBSERVATION

The sample colouration is _____ than the standard colouration.

RESULT

The given sample of sodium chloride _____ the limit test for heavy metals.